10 Models — What Comes after the Next Generation?

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CONTENTS

10.1 INTRODUCTION

This chapter will highlight two separate and generally unrelated areas of predictive food microbiology: models for cross-contamination and inoculum size (or models that consider the initial number of organisms present). These two classes of models are being included together here, because they represent some of the newer areas of predictive modeling that are less well developed compared to the more well-known and established research areas such as growth and inactivation modeling. This chapter will summarize the current state of research in these two rapidly evolving areas and will conclude with a short example describing preliminary investigations into the integration of these two fields of study.

10.2 CROSS-CONTAMINATION

Predictive models for microbial behavior have traditionally focused on describing increasing concentrations (as a result of multiplication) and decreasing concentrations (as a result of cell death). A number of lines of inquiry have pointed out the need for a third class of models that may be required in some cases: cross-contamination models. The three lines of inquiry all come from the interaction of risk assessment and epidemiology, and are related to three very different microorganisms: *Listeria monocytogenes*, *Campylobacter jejuni*, and food-borne viruses.

L. monocytogenes is a psychrotrophic pathogen that can cause mild illness in healthy adults and spontaneous abortion in pregnant women. The organism is easily destroyed by heating but readily recontaminates the cooked product prior to packaging.[25] While this recontamination is known to take place, (and can contribute to significant disease outbreaks) very few mathematical models are available for microbial risk assessors to use.

The situation with *Campylobacter* is slightly different. In this case, the organism is known to cause cross-contamination in a significant number of cases[1] but the means by which this occurs is not clear.[11] Risk assessment models for Campylobacteriosis have incorporated cross-contamination events during final preparation in a kitchen environment, but as with *Listeria* risk assessment models, few suitable models are available.

Finally, it is known that a number of foodborne disease agents (primarily viruses) can contaminate foods, and in many cases the source of the agent has been an ill food worker.[12] While quantitative microbial risk assessments have yet to address ill workers, hand-to-food and other cross-contamination rates using a nonpathogenic surrogate have been calculated with sufficient detail to be suitable for risk assessment.[7]

10.2.1 Issues of Concern

There are a number of issues of particular concern that are important in modeling cross-contamination — issues that are unique to these sorts of models as compared to the traditional growth and decline models: appropriate statistical treatment of data, transfer from one location to another, factors influencing transfer, and the possibility of multiple transfers.

10.2.1.1 Statistical Treatment

Data on cross-contamination are typically presented as percent transfer, as shown below:

$$\frac{\text{CFU on target}}{\text{CFU on source}} * 100 = \text{percent transfer}$$

The problem arises when multiple observations of the same conditions are to be combined and reported as an average. It has been shown that when large numbers of observations are made, the distribution of "percent transfer rates" is distinctly

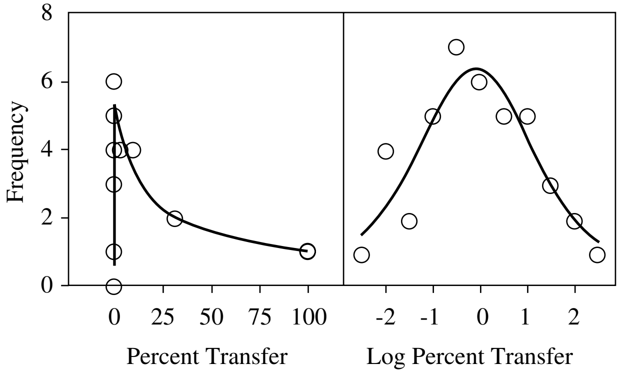


FIGURE 10.1 Distribution of percent transfer data with linear (left panel) and logarithmic transformation (right panel).

nonnormal.[7] This point is made clearer when the data are presented visually. Figure 10.1 shows the same data set plotted as number of observations vs. percent transfer (left panel) and number of observations vs. $\log_{10}$ percent transfer (right panel). This same pattern is borne out for many different types of surface-to-surface transfers.[7,10] Since percent transfer is nonnormally distributed, but $\log_{10}$ percent transfer is approximately normally distributed, this means data should be log transformed before averages are calculated. This apparently subtle distinction has important consequences as illustrated below.

Let us assume we have two observed transfer rates of 5 and 50%. If the mean is calculated arithmetically:

$$(50 + 5)/2 = 27.5\% \text{ transfer}$$

Alternatively, using the statistically appropriate $\log_{10}$ transformed rates leads to a more complex series of calculations:

$$(\log 0.05 + \log 0.50)/2 = (-1.30103 + -0.30105)/2 = -0.80103$$

Then this number should be converted back to the untransformed percent scale:

$$10^{-0.80103} = 15.8\% \text{ transfer}$$

So this simple difference leads to a calculated transfer rate that is over half that obtained when the statistically incorrect method is used.

10.2.1.2 Additive Nature of Cross-Contamination

Another key feature of any cross-contamination model is that some consideration should be made of both the source of the contaminant and the destination. If the source is a contaminated surface, then the number of organisms on that surface must

be known, since (at least in theory) a more contaminated source will yield a more contaminated destination. Care must be taken to ensure that the number of organisms to be added to an already contaminated item is added in a numerically correct manner. For example, most calculations in a microbial risk assessment will be $\log_{10}$ CFU increases or decreases. This would not be correct in cross-contamination. If a food contained 10 organisms and 100 were added, this would not be a two $\log_{10}$ increase, 1 + 2 = 3 or 1000 organisms, but 10 + 100 = 110 organisms.

10.2.1.3 Factors Influencing Transfer Rate

The next issue that should be addressed would be a consideration of the factors influencing transfer rate. There are a whole host of factors that may influence transfer, like source (air, liquid, or solid), the pressure applied (for solid-to-solid transfers), menstrum effects, contact time, number of organisms present, and surface characteristics. Some of these factors have been investigated for transfer from hands in a healthcare setting, but little data applicable to food systems have been published. What has been published in the food and healthcare literature has not been systematic or comprehensive.[21]

10.2.1.4 Types of Transfers

A recent review[10] describes some of the currently available models for recontamination via air, via processing equipment (i.e., biofilms), or via hand contact. These authors point out that not many available models are directly applicable to the food industry as most models are developed for aquatic or environmental systems. In some cases currently available models are contradictory or incompatible. For example, competing air recontamination models assume that when the concentration in the air increases linearly, the concentration in the product increases either linearly[28] or quadratically.[23] The implications of such assumptions obviously have a critical impact on model predictions.

Models for recontamination that consider the effect of biofilms are quite well developed, not because of their importance in food processing, but because of their application in wastewater treatment. Biofilm models can be one-dimensional, or multidimensional,[16] but the key feature of any biofilm model used for food recontamination is not its dimensionality, but its ability to consider attachment, growth, and detachment averaged over the food contact surface.[10] Some biofilm models appropriate for use in food systems have been developed.[31]

den Antrekker et al.[10] conclude their review by proposing a schematic for a general contamination model that is suitable for modeling recontamination via air, via surfaces, or via hands. This model uses a source, an intermediate phase, and a product, with transfer rates between source and intermediate phases and intermediate and product phases that govern the overall transfer to the product.

10.2.1.5 Multiple Transfers and Complexity

The last issue of concern is the modeling of multiple transfers. The food preparation or handling environment may be such that multiple transfers between many different

environments may occur. In a processing plant it may be from air to surface, surface to product, product to clean surface, and finally surface to clean product. In a food service environment it may be from a contaminated surface to workers' hands, and then from hands to food. In a home environment it may be from a contaminated food product to a surface, and then from that surface to another food.

Each of these simple examples would require a series of calculations, using models that generally do not yet exist. At the same time, it should be realized that the real world is considerably more complex than these simple examples show, and that there may be literally dozens of cross-contamination possibilities in even a simple food process or meal preparation.

10.2.2 Cross-Contamination Summary

The development of mathematical models suitable for describing cross-contamination events in food production, processing, and preparation is still in its infancy, and might be likened to the general state of predictive food microbiology in the 1980s. The past two decades have seen many improvements in the general state of the art of predictive food microbiology, and there is no reason to believe the next two decades would not experience similar improvements in cross-contamination modeling. This field has attracted the interest of a number of research groups around the world, and the beginnings of a comprehensive body of work are beginning to emerge.

10.3 INOCULUM SIZE MODELING

Predictive models have traditionally been developed using starting bacterial concentration that may be quite high relative to the levels found in some foods. Modelers developing models in this way were quite justified in their choice of this approach. In some cases, inoculum size does not have a significant effect on the response to be modeled (i.e., growth rate).[5] High initial inoculum size also represents a conservative worst-case approach to modeling, and these high starting concentrations helped to assure repeatability and simplified some of the considerations about microbial variability, often called "biovariability."

Despite the logic seen in this worst-case approach, modelers have always sought to improve their models by making them more realistic and representative of real-world conditions. Also, as predictive food microbiologists' modeling tools and abilities have improved, their ability to handle more complex models and modeling techniques have improved concomitantly. As part of this evolutionary improvement in modeling ability, some modelers have sought to address this shortcoming by developing models that take initial microbial concentrations into consideration.

10.3.1 *Clostridium botulinum*

One of the earliest examples of a predictive model that explicitly acknowledged the influence of inoculum size were models developed for *C. botulinum.*[13,14] The authors' specific objective in this case was to develop models capable of predicting the probability of toxin formation from a single *C. botulinum* spore. Later research in

this same lab also showed an inoculum size effect in fish[3] and poultry[19] systems inoculated with *C. botulinum*.

The importance of inoculum size for models for *C. botulinum* has since been well documented in the literature.[17,26,27,29,30] These *C. botulinum* models were developed as an aid to the food industry, but also served to point the way towards a more mechanistic understanding of populations of *C. botulinum* spores. The model for nonproteolytic *C. botulinum* developed by Whiting and Oriente,[26] for example, showed that not only did lower population of spores exhibit longer time-to-turbidity, the variability around that time increased markedly with decreasing inoculum size as well. This same effect was also seen with proteolytic *C. botulinum* spores.[27] These results supported the observations made by others conducting microscopy studies that there was a marked variability seen in the germination, outgrowth, and lag time in individual *C. botulinum* spores observed directly.[4] These apparent interactions between spores of *C. botulinum* have also been demonstrated using computer simulation of different inoculum sizes[30] and seem to be caused by the release of a signaling molecule into the culture media by germinating spores.

This inoculum phenomenon does not appear to be unique to *C. botulinum*, as it has also been observed in *Bacillus cereus*[9] and *Bacillus megaterium*[6] using direct microscopic observation and in *Bacillus stearothermophilus*[15] indirectly by time to turbidity.

10.3.2 Nonspore-Forming Bacteria

The effect has also been seen in models for nonspore-forming bacteria, like *Brochothrix thermosphacta*,[18] as well as in nonmodeling research with *Salmonella*[8] and *L. monocytogenes.*[2,22,24] These publications show that the effect is most pronounced when cells are stressed[2,24] or cultured in inhospitable environments.[8] The response in vegetative cells has been interpreted by some as being due to death of a proportion of cells in the inoculum rather than communication, as appears to be the case with *C. botulinum,*[22] although others have shown that addition of spent medium from a stationary-phase culture reduces the variability and length of lag times.[24]

10.3.3 Inoculum Size Summary

Clearly modelers have moved beyond predictive models developed using high initial bacterial concentrations to models using a range of contamination levels. Most of the effort in this area has focused on models for spore-forming organisms, specifically *C. botulinum*. A limited amount of work has also been done with other spore-forming organisms and vegetative cells. It appears that inoculum size has the most dramatic effect on the lag time (for vegetative cells) or germination, outgrowth, and lag time (for spores).

10.4 CROSS-CONTAMINATION AND INOCULUM SIZE

While the two subjects of this chapter do not appear to have much in common, except for both being aspects of modeling on the "cutting edge," there appears to

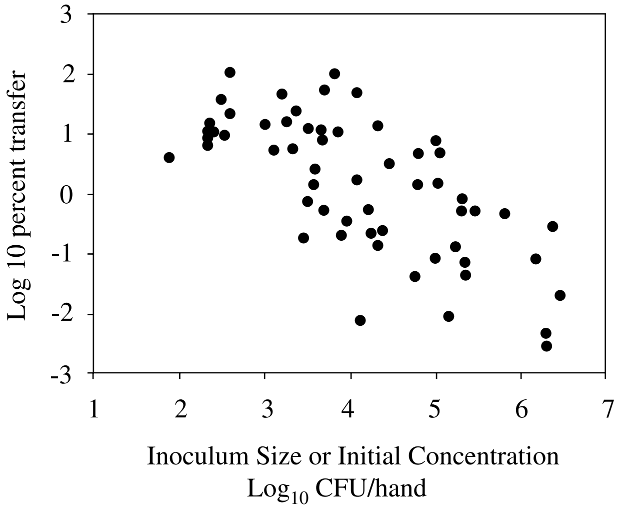


FIGURE 10.2 Percent transfer as a function of inoculum size.

be some evidence that inoculum size is not only important in traditional growth modeling, but that it may also be an important consideration in modeling cross-contamination. Figure 10.2 presents a reanalysis of a portion of the data originally published by Chen et al.[7] for cross-contamination rate from bare hands contaminated with *Enterobacter aerogenes* to lettuce. While the r^2 value (0.49) and a visual inspection of the plot show that the correlation is not ideal, the effect is highly significant ($p > 10^{-10}$). The regression model for the plot indicates that changing the starting concentration by 1.5 $\log_{10}$ CFU will change the $\log_{10}$ percent transfer rate by about 1 (i.e., from 10 to 1%). It is also interesting to note that the relationship between starting concentration and $\log_{10}$ percent transfer rate is an inverse one, so that as the starting concentration decreases, the transfer rate increases. This could have very profound food safety consequences since low levels of pathogens would have a correspondingly greater ability to transfer. This simple example has been used to illustrate the exciting and complex nature of predictive food microbiology at the expanding edges of the discipline.

10.5 SUMMARY

Models for cross-contamination and inoculum size represent areas at the expanding edge of predictive food microbiology. Both areas characterize modelers' attempts to make models more useful and representative of microbial behaviors seen in the real world. Both areas continue to present mathematical, statistical, and methodological challenges to those working in the field.

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